

What is claimed is:

1. A clostridial neurotoxin substrate comprising
5 any peptide or protein that can serve as a substrate
for the proteolytic activity of any clostridial
neurotoxin, said protein or peptide having been
modified to contain a signal moiety on one side of the
cleavage site, and a moiety on the other side of the
10 cleavage site that quenches the magnitude of that
signal such that when the substrate is cleaved, an
increase in signal is produced.

2. The substrate according to claim 1 wherein
15 said clostridial neurotoxin is botulinum neurotoxin
serotype A.

3. The substrate according to claim 2 wherein
said substrate is a peptide identified in SEQ ID NO:1
20 or SEQ ID NO:2.

4. The substrate according to claim 1 wherein
said clostridial neurotoxin is botulinum neurotoxin
serotype B or tetanus toxin.
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5. The substrate of claim 4 wherein said
substrate is identified in SEQ ID NO:3 and SEQ ID
NO:4.

6. The substrate according to claim 1 wherein
30 said clostridial neurotoxin is botulinum neurotoxin
serotype D or botulinum neurotoxin serotype F.

7. The substrate of to claim 6 wherein said
35 substrate is chosen from the group consisting of a

peptide identified in SEQ ID NO:5, SEQ ID NO:6, and
SEQ ID NO:7.

8. A method for detecting the presence of
5 clostridial neurotoxin proteolytic activity in a
sample said method comprising
mixing the sample with a peptide substrate
according to claim 1, and
detecting an increase in signal produced from
10 proteolytic cleavage of said substrate.

9. A method for measuring concentration of
neurotoxin in a sample, comprising
mixing the sample with a peptide substrate
15 according to claim 1,
measuring an increase in signal with time
produced from proteolytic cleavage of said substrate
and,
determining the concentration of said neurotoxin
20 by correlation to a standard.

10. The method according to claim 9 wherein said
neurotoxin is botulinum neurotoxin serotype A.

25 11. The method according to claim 10 wherein said
peptide substrate is a peptide identified in SEQ ID
NO:1 or SEQ ID NO:2.

12. The method according to claim 9 wherein said
30 neurotoxin is botulinum neurotoxin serotype B or
tetanus toxin.

13. The method according to claim 12 wherein said
peptide substrate is a peptide identified in SEQ ID
35 NO:3 or SEQ ID NO:4.

14. The method according to claim 9 wherein said neurotoxin is botulinum neurotoxin serotype D or F.

5 15. The method according to claim 14 wherein said peptide substrate is chosen from the group consisting of a peptide identified in SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

10 16. A kit for determining the concentration of a clostridial neurotoxin in a sample, the kit containing in close confinement,

- (i) one or more peptide substrates according to claim 1 cleavable by said clostridial neurotoxin;
- 15 (ii) said clostridial neurotoxin standard.

17. The kit according to claim 16 wherein said clostridial neurotoxin is botulinum neurotoxin serotype A and the peptide substrate is one or both of
20 the peptides identified in SEQ ID NO:1 and SEQ ID NO:2.

18. The kit according to claim 16 wherein said clostridial neurotoxin is botulinum neurotoxin
25 serotype B or tetanus toxin and the peptide substrate is one or both of the peptides identified in SEQ ID NO:3 and SEQ ID NO:4.

19. The kit according to claim 16 wherein said
30 clostridial neurotoxin is botulinum neurotoxin serotype D or F and the peptide substrate is one or more of the peptides identified in SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7.

20. A botulinum neurotoxin substrate comprising any peptide or protein that can serve as a substrate for the proteolytic activity of any clostridial neurotoxin, said protein or peptide having been
5 modified so that it can be attached on one side of the proteolytic cleavage site to a solid material.

21. The substrate according to claim 20 wherein said clostridial neurotoxin is botulinum neurotoxin
10 serotype A.

22. The substrate according to claim 21 wherein said substrate is a peptide identified in SEQ ID NO:8 or SEQ ID NO:11.
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23. The substrate according to claim 20 wherein said clostridial neurotoxin is botulinum neurotoxin serotype B or tetanus toxin.

20 24. The substrate of claim 23 wherein said substrate is identified in SEQ ID NO:9.

25 25. The substrate according to claim 20 wherein said clostridial neurotoxin is botulinum neurotoxin serotype D or serotype F.

26. The substrate of claim 25 wherein said substrate is a peptide identified in SEQ ID NO:10.

30 27. The substrate of claim 20 wherein said botulinum neurotoxin is botulinum neurotoxin serotype E.

28. The substrate of claim 27 wherein said substrate is a peptide identified in SEQ ID NO:11 and 12.

5 29. A method for detecting the presence of clostridial neurotoxin proteolytic activity in a sample said method comprising

 mixing the sample with a peptide substrate according to claim 20, and

10 detecting an increase in signal produced from proteolytic cleavage of said substrate.

 30. A method for measuring concentration of neurotoxin in a sample, comprising

15 mixing the sample with a peptide substrate according to claim 20,

 measuring an increase in signal with time produced from proteolytic cleavage of said substrate and,

20 determining the concentration of said neurotoxin by correlation to a standard. .

 31. The method according to claim 30 wherein said neurotoxin is botulinum neurotoxin serotype A.

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 32. The method according to claim 31 wherein said peptide substrate is a peptide identified in SEQ ID NO:8 or SEQ ID NO:11.

30 33. The method according to claim 30 wherein said neurotoxin is botulinum neurotoxin serotype B or tetanus toxin.

34. The method according to claim 33 wherein said peptide substrate is a peptide identified in SEQ ID NO:9.

5 35. The method according to claim 30 wherein said neurotoxin is botulinum neurotoxin serotype D or F.

36. The method according to claim 35 wherein said peptide substrate is a peptide identified in SEQ ID
10 NO:10.

37. The method according to claim 30 wherein said neurotoxin is botulinum neurotoxin serotype E.

15 38. The method according to claim 37 wherein said peptide substrate is a peptide identified in SEQ ID NO:11 or SEQ ID NO:12.

39. A kit for determining the concentration of a
20 clostridial neurotoxin in a sample, the kit containing in close confinement,

(i) one or more peptide substrates according to claim 20 cleavable by said clostridial neurotoxin;

(ii) said clostridial neurotoxin standard.

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40. The kit according to claim 39 wherein said clostridial neurotoxin is botulinum neurotoxin serotype A and the peptide substrate is one or both of the peptides identified in SEQ ID NO:8 and SEQ ID
30 NO:11.

41. The kit according to claim 39 wherein said clostridial neurotoxin is botulinum neurotoxin serotype B or tetanus toxin and the peptide substrate
35 is a peptide identified in SEQ ID NO:9.

42. The kit according to claim 39 wherein said
clostridial neurotoxin is botulinum neurotoxin
serotype D or F and the peptide substrate is a peptide
5 identified in SEQ ID NO:10.

43. The kit according to claim 39 wherein said
clostridial neurotoxin is botulinum neurotoxin
serotype E and the peptide substrate is one or more of
10 the peptides identified in SEQ ID NO:11 or SEQ ID
NO:12.

44. A method for identifying inhibitors or
enhancers of proteolytic activity of a clostridial
15 neurotoxin comprising:
preincubating a neurotoxin with a test compound
to make a neurotoxin-compound solution,
exposing said solution to a substrate of said
neurotoxin according to claim 20,
20 measuring signal resulting from the proteolysis
of said substrate by said neurotoxin, and
comparing said signal with controls,
wherein an increase in signal indicates a
compound which enhances neurotoxin activity and a
25 decrease in signal indicates a compound which inhibits
said neurotoxin.

45. The method according to claim 44 wherein said
neurotoxin is botulinum neurotoxin serotype A and the
30 substrate is a peptide identified in SEQ ID NO:8 or
SEQ ID NO:11.

46. The method according to claim 44 wherein said
neurotoxin is botulinum neurotoxin serotype B or

tetanus toxin and the substrate is a peptide identified in SEQ ID NO:9.

47. The method according to claim 44 wherein said
5 neurotoxin is botulinum neurotoxin serotype D or F and the substrate is a peptide identified in SEQ ID NO:10.

48. The method according to claim 44 wherein said
neurotoxin is botulinum neurotoxin serotype E and the
10 substrate is one or more of the peptides identified in SEQ ID NO:11 or SEQ ID NO:12.

49. A method for identifying a serotype of a
clostridial neurotoxin in a sample suspected of
15 containing a neurotoxin, the method comprising
incubating the sample with antibodies against
each clostridial neurotoxin such that a neurotoxin is bound to its serotype-specific antibody,
removing unbound components,
20 adding activation solution such that clostridial protease is activated,
adding solutions containing clostridial neurotoxin peptide substrates according to claim 1 to said activated protease,
25 detecting signal generated from proteolysis of said substrate by said protease, wherein a signal above control indicates presence of a neurotoxin, and
determining the serotype of the clostridial neurotoxin by noting the specificity of the antibody.

30 50. The method of claim 49 wherein the antibodies are bound to a solid material.

51. The method of claim 50 wherein the solid
35 material is a multiwell plate.

52. The method of claim 51 wherein each well contains an antibody specific for a different neurotoxin serotype.

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53. The method of claim 49 wherein each peptide substrate is labeled with a different signal.

54. A kit for identifying a serotype of a
10 clostridial neurotoxin in a sample suspected of
containing a neurotoxin, comprising
serotype-specific antibodies
clostridial neurotoxin standards, and
peptide substrates according to claim 1.

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